



THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re PATENT APPLICATION OF: Cox, et al.)	Group Art Unit: 1646
)	
U.S. Serial No.: 10/731,411)	Examiner: Daniel E. Kolker, Ph.D.
)	
Filed: December 8, 2003)	
)	
For: NEUROTOXIC AMINO ACID OR)	
NEUROTOXIC DERIVATIVE THEREOF)	
ASSOCIATED WITH NEUROLOGICAL)	
DISORDERS)	

DECLARATION OF DR. SANDRA BANACK UNDER 37 C.F.R. § 1.132

Assistant Commissioner for Patents
U.S. PATENT AND TRADEMARK OFFICE
Box 1450
Alexandria, VA 22313-1450

Sir:

I, the undersigned Sandra Banack, Ph.D., do hereby declare:

1. I am a co-inventor of the subject matter claimed in the above-identified United States Patent Application, Serial No. 10/731,411, filed at the United States Patent and Trademark Office on December 8, 2003.
2. I am currently an Associate Professor of Biological Science at California State University, Fullerton and have held this position since 2002. From 1997 to 2002, I was an Assistant Professor at California State University, Fullerton. I am also a senior scientist at the Institute for Ethnomedicine in Provo, Utah. I was an Umeå Research Fellow at Umeå University, Sweden in 1996 and a Visiting Assistant Professor of Botany at Brigham Young University from 1994-1997. I obtained a Bachelor of Science degree in Zoology and a Master of Science degree in Botany from Brigham Young University in

1985 and 1987 respectively. I completed a Ph.D. at the University of California, Berkeley in Integrative Biology in 1996. My *curriculum vitae* is attached.

3. I have reviewed the claims that are presently under examination.
4. I have read the Office Action mailed December 2, 2004, in the above-identified application, and am familiar with the Examiner's rejection of the claims.
5. I submit this declaration in support of the fact that BMAA can be measured in non-neurological tissues from living subjects, and furthermore, that detectable amounts of BMAA can be measured in tissues from living subjects having a neurological disorder.
6. The following study, performed by me or under my direction, employed compositions and techniques very similar to those disclosed in the specification in Example 4 to determine total BMAA in tissue.
7. Briefly, hair samples were taken from human subjects living in North America, and BMAA levels in the hair samples were measured as described below. Each hair sample was weighed, hydrolyzed in 6.0 M sodium hydroxide for 12-18 hours, and then filtered through a 0.2 micron filter. Each filtrate was dried in a vacuum centrifuge, reconstituted in borate buffer (0.5M), and then derivatized with 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (ACQ). BMAA levels were measured in each hair sample processed as described above, using HPLC techniques as described in the specification, with minor modifications. BMAA was separated from other protein amino acids by reverse phase elution (Waters Nova-Pak C₁₈ column, 300 mm x 3.9 mm) with 140 mM sodium acetate, 5.6 mM triethylamine, pH 5.2 (mobile phase A) and 52% acetonitrile in water (mobile phase B) at 37°C. The standard amino acid elution gradient was modified for BMAA separation (0.0 min = 100% A , 2.0 min = 90% A curve 11, 5.0 min = 86% A curve 11, 10.0 min = 86% A curve 6, 18.0 min = 73% A curve 6, 30.0 min = 60% A curve 10, 35.0 min = 40% A curve 6, 38.0 min = 100% B, 45 minutes = 100% A). BMAA concentration was determined by detection of the fluorescent tag using a Waters 2487 Dual-λ Fluorescence Detector, with excitation at 250 nm and emission at

395 nm. BMAA levels were determined by comparison with a BMAA standard at a concentration representing a single mid-range concentration as described in the specification. The results were expressed as micrograms of BMAA per gram of hair ($\mu\text{g/g}$). The limit of detection was $0.000065 \mu\text{mol}$ per injection and the limit of quantification was $0.0065 \mu\text{mol/injection}$.

8. Exhibit 2, submitted herewith, is a table showing the results from our measurements of total BMAA levels in hair samples from 18 living human subjects. Hair samples from the 9 subjects having clinical diagnoses of Alzheimer's disease, Alzheimer's type dementia, pre-Alzheimer's vascular dementia, or dementia, had a mean BMAA level of $21.4 \mu\text{g/g}$ of hair, where individual samples had levels ranging from $4.0 \mu\text{g/g}$ to $47 \mu\text{g/g}$. Hair samples from the 9 living subjects that did not have any of the before-mentioned diagnoses, described as "control" subjects in Exhibit 2, had no detectable BMAA.
9. I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

By:

Sandra Bamaek

Date:

25 May 2005



SANDRA ANNE BANACK
Curriculum Vitae

Degrees

Ph.D. (Integrative Biology)	University of California, Berkeley	1996
M.S. (Botany)	Brigham Young University	1987
B. S. (Zoology)	Brigham Young University	1985

Academic and Post-doctoral Experience

Associate Professor (Biology)	California State University, Fullerton	2002-present
Assistant Professor (Biology)	California State University, Fullerton	1997-2002
Umeå Research Fellow	Umeå University, Sweden	1996
Visiting Assistant Professor (Botany)	Brigham Young University	1994-1997

Publications

Peer-reviewed Journals

- 1) Cox, P.A., Banack, S.A., Murch, S.J, Rasmussen, U., Tien, G., Bidigare, R.R., Metcalf, J.S., Morrison, L.F., Codd, G.A., Bergman, B. (2005). Diverse taxa of cyanobacteria produce B-N-methylamino -L-alanine, a neurotoxic amino acid. *Proceedings of the National Academy of Sciences* 102(14): 5074-5078.
- 2) Murch, S.J., Cox, P.A., Banack, S. A., Steele, J.C., Sacks, O.W. 2004. Occurrence of β -methylamino-L-alanine (BMAA) in ALS/PDC patients from Guam. *Acta Neurologica Scandinavica* 110:267-269.
- 3) Murch, S.J., Cox, P.A., Banack, S.A. 2004. A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. *Proceedings of the National Academy of Sciences* 101(33): 12228-12231.
- 4) Banack, S. A, X. Rondon, and W. Diaz-Huamanchumo. 2004. Indigenous Cultivation and Conservation of Totora (*Schoenoplectus californicus*, Cyperaceae) in Peru. *Economic Botany* 58: 11-20.
- 5) Walker, M., J. Nunez, M. Walkingstick, and S.A. Banack. 2004. Ethnobotanical investigation of Acjachemen clapperstick from blue elderberry (*Sambucus mexicana*). *Economic Botany* 58: 21-24.
- 6) Eiler, K. C., and S. A. Banack. 2004. Variability in the alarm call of the golden-mantled ground squirrels (*Spermophilus lateralis* and *S. saturatus*). *Journal of Mammalogy*. 85(1):43-50.
- 7) Cox, P. A. and Banack, S. A. Flying Fox Pollination. *Scientific American*. *In press*.
- 8) Cox, P. A., Banack, S. A. & Murch, S. J. 2003. Biomagnification of Cyanobacterial Neurotoxins and neurodegenerative disease among the Chamorro people of Guam *Proc. Natl. Acad. Sci. USA* 100 (23): 13380-13383.

- 9) Banack S. A. and Cox, P. A. 2003. Distribution of the neurotoxic non-protein amino acid BMAA in *Cycas micronesica*. Botanical Journal of the Linnean Society, 143: 165-168.
- 10) Banack, S. A. and Cox, P. A. 2003. Biomagnification of cycad neurotoxins in flying foxes: implications for ALS-PDC in Guam. Neurology 61:387-389.
- 11) Monson, C.S., Banack, S. A., and Cox, P. A. 2003. Conservation implications of Chamorro consumption of flying foxes as a possible cause of amyotrophic lateral sclerosis-parkinsonism dementia complex in Guam. Conservation Biology. 17: 678-686.
- 12) Rondon, X. J., S. A. Banack, W. Diaz-Huamanchumo. 2003. Ethnobotanical investigation of caballitos (*Schoenoplectus californicus*: Cyperaceae) in Huanchaco, Peru. Economic Botany 57:35-47.
- 13) Banack, S. A. and G. S. Grant. 2003. Spatial and temporal movement patterns of the flying fox, *Pteropus tonganus*, in American Samoa. Journal of Wildlife Management 66:1154-1163.
- 14) Banack, S. A. and G. S. Grant. 2003. Reproduction and behaviour of the Samoan flying fox, *Pteropus samoensis* (Chiroptera, Pteropodidae). Mammalia. 67:419-437.
- 15) Banack, S. A., M. H. Horn, and A. Gawlicka 2002. Disperser-vs. establishment-limited distribution of a riparian fig tree (*Ficus insidida*) in a Costa Rican Tropical Rain Forest. Biotropica 34:232-243.
- 16) Banack, S. A. 2001. *Pteropus samoensis*. Mammalian Species Account 661: 1-4.
- 17) Grant, G. S. and S. A. Banack. 1999. Reproductive biology in *Pteropus tonganus*. Australian Mammalogy. 21: 111-120.
- 18) Richmond, J. Q., S. A. Banack, and G. S. Grant. 1998. Comparative analysis of wing morphology, flight behaviour, and habitat use in flying foxes (Genus: *Pteropus*). Australian Journal of Zoology. 46: 283-289.
- 19) Banack, S. A. 1998. Diet selection and resource use by flying foxes (Genus *Pteropus*). Ecology. 79 (6): 1949-1967.
- 20) Grant, G. S. and S. A. Banack. 1995. Predation on *Pteropus tonganus* by a Barn Owl in American Samoa. Australian Mammalogy 18:48-50.
- 21) Grant, G. S., S. A. Banack, and P. Trail. 1994. Decline of the Sheath-tailed bat *Emballonura semicaudata* (Chiroptera: Emballonuridae) on American Samoa. Micronesia 27(1\2):133-137.
- 22) Banack, S. A. and P. A. Cox. 1987. Ethnobotany of Ocean-Going Canoes in Lau, Fiji. Economic Botany 41:148-162

Book:

- 23) Cox, P. A. and S. A. Banack (eds.). 1991. Islands, Plants, and Polynesians. Dioscorides Press: Portland, Oregon.

Book Chapter:

- 24) Banack, S. A. 1991. Plants and Polynesian voyaging. IN Cox, P.A. and S.A. Banack (eds.) Islands, Plants, and Polynesians. Dioscorides Press: Portland, Oregon.

Book Reviews:

- 25) Banack, S. A. 2002. Flying foxes: fruit and blossom bats of Australia. Quarterly Review of Biology. 215-216.
26) Banack, S. A. 1997. Ethnobotany, Principles and Applications. Plant Science 130:117-118.
27) Banack, S. A. 1997. Plants, People, and Culture. Economic Botany 51:191-192.

Scholarships and Fellowships

NTBG Research Fellow	National Tropical Botanical Garden	2000-present
Umea Post-Doctoral Fellowship	Umea University	1996
Graduate Division Fellowship	University of California, Berkeley	1995
Berkeley Joseph Maillard Fellowship	Museum of Vertebrate Zoology	1992-1993
Academic Scholarship	Mobil Oil Canada	1981-1984
Jennie Clyde Scholarship	Brigham Young University	1984-1985
Marigold N. Saunders Scholarship	Brigham Young University	1982-1984
Presidential Scholarship	Brigham Young University	1981-1982
Scholastic Award	Saskatchewan Provincial Gov.	1981
Biology Scholarship	Swift Current, Saskatchewan	1981

Honors

Outstanding Scholarship	Calif. State Univ.	2004
Outstanding Contributions to Student Learning	Calif. State Univ.	2002
Julia F. Morton Award	Society for Economic Botany	2000, 2001
Bacardi Student Award	Lubee Foundation	1995
Outstanding Graduate Student Instr.	University of California, Berkeley	1992
Phi Kappa Phi		1987
Phi Eta Sigma		1982

Grants

Bioaccumulation of BMAA	California State Mini Grant	2004
Ethnobotany / Medicinal Plants	NIH planning Grant (Collaborator)	2003
ALS/PDC Research	National Tropical Botanical Garden	2002 - 2005
ALS and flying foxes	ALS Association	2002
Biomagnification of BMAA	California State Mini Grant	2002
Flying Fox Research	CSUF Undergraduate Research Support	2000, 2002
Environmental Biol / So Cal	NSF / UMEB (Collaborator)	2001
Vertebrate Ecology / So Cal	California State Mini Grant	2000
Vertebrate Research	CSUF Untenured Faculty Grant	2000
Service Learning Grant.	California State University	2000, 2001
Achachemen Ethnobotany	Undergraduate Research Support	2000

Peruvian Ethnobotany	Untenured Faculty Grant	1998, 1999
Vertebrate Ecology	California State Mini Grant	1999
Curriculum Development	NSF (Co-PI)	1999
Vertebrate Ecology / So Cal	California State Mini Grant	1997
Vertebrate Ecology / So Cal	Untenured Faculty Grant	1997
Ecology and Ethnobotany	California State University	1997-1998

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 Filed: December 8, 2003

EXHIBIT 2. BMAA LEVELS IN HAIR SAMPLES

Diagnosis	BMAA (µg/g)
Alzheimer's	11.9
Alzheimer's	4.0
Alzheimer's type dementia	13.2
Alzheimer's type dementia	47.4
Alzheimer's	44.4
Pre-Alzheimer's vascular dementia	32.3
Alzheimer's type dementia	9.8
Alzheimer's type dementia	19.6
Dementia	10.1
Alzheimer's Mean	21.4
Control	ND
Control	ND
Control	ND
Control	ND
Control	ND
Control	ND
Control	ND
Control	ND
Control	ND
Control Mean	0.0